Journal of Fish Diseases 2009, **32,** 401–408 doi:10.1111/j.1365-2761.2009.01024.x

Evaluation of diquat against an acute experimental infection of *Flavobacterium columnare* in channel catfish, *Ictalurus punctatus* (Rafinesque)

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Abstract

A trial was performed to evaluate the efficacy of diquat (6,7-dihydrodipyrido[1,2-a:2′,1′-c]pyrazinediium dibromide) against an acute experimental infection of *Flavobacterium columnare* in channel catfish, *Ictalurus punctatus*. Diquat is an Environmental Protection Agency-approved herbicide and has the potential to be legally and practically used against columnaris. Channel catfish were challenged, by cutaneous abrasion, and waterborne exposure to *F. columnare* and treated once at 22-h post-challenge with 2.5, 5.0, 10.0 and 15 mg L^{-1} of diquat active ingredient for 6 h. At the conclusion of the trial, 21-day post-challenge, diquat at 5.0, 10.0 and 15 mg L^{-1} significantly ($P < 0.05$) reduced the mortality of infected fish from 95% in the challenged non-treated fish to 68%, 59% and 49%, respectively. *In vitro*, the minimum inhibitory concentration (MIC) of 23 isolates of *F. columnare* was assayed. The majority of the isolates had an MIC value of 5 μg mL^{-1} (15 of the 23 isolates). Infected fish exhibited acute clinical signs similar to a natural infection. The skin had severe ulcerative necrotizing dermatitis and the muscles had severe necrotizing myositis. The gills had severe multifocal necrotizing branchitis. The results demonstrate that diquat would reduce mortalities caused by an acute columnaris infection.

Keywords: channel catfish, columnaris, diquat, efficacy, *Flavobacterium columnare*.

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Introduction

Columnaris disease exists worldwide and affects a wide variety of fish species. In fact, no wild or cultured freshwater fish, including ornamental fish in tanks, are known to be totally resistant to columnaris (Plumb 1999). Columnaris is one of the two most costly diseases to the US channel catfish, *Ictalurus punctatus* (Rafinesque), industry (Wagner, Wise, Khoo & Terhune 2002). The disease has been reported to cause mortalities >90% in salmonid and eel populations and in tank-held channel catfish (Hussain & Summerfelt 1991; Plumb 1999). *Flavobacterium columnare*, the aetiological agent of columnaris, causes a combination of external and systemic infection (Hawke & Thune 1992). Although *F. columnare* can be a primary pathogen, it is more commonly a secondary pathogen requiring predisposing stress or trauma to the host. Channel catfish are susceptible to columnaris at temperatures from 15 to 30 °C and young fish are more severely affected than adults (Plumb 1999). The bacteria attack the fins, skin and gills of fish. Clinical signs of columnaris include frayed necrotic fins with greyish to white margins, depigmented and necrotic skin, white to brown necrotic areas in the gills while viscera exhibit little or no pathology even when the infection is systemic (Plumb 1999).

Diquat (6,7-dihydrodipyrido[1,2-a:2′,1′-c]pyrazinediium dibromide) is an Environmental Protection Agency-approved herbicide for use in food fish ponds (Plumb 1999) and has the potential to be legally and practically used for treating columnaris infections (Thomas-Jinu & Goodwin 2004). Diquat forms a stable free radical that reacts with

oxygen to generate superoxide. Superoxide can generate hydrogen peroxide, which can directly cause toxic oxidation. The hydrogen peroxide and superoxide can attack polyunsaturated lipids present in cell membranes to produce lipid hydroperoxides; these products can attack other unsaturated lipids to form additional lipid free radicals (Timbrell 1989). Noga (1996) and Plumb (1999) have reported that diquat has efficacy against external bacterial infections, especially bacterial gill disease and columnaris. Plumb (1999) recommended 0.25–2.5 mg L^{-1} indefinitely or 2–4 mg L^{-1} for 1 h as a prolonged bath. Thomas-Jinu & Goodwin (2004) conducted the only controlled study that examined the efficacy of diquat against experimental columnaris. The results of the study clearly demonstrated the efficacy of diquat as an indefinite treatment at a concentration of 5.4 mg L^{-1}; treated fish had 100% survival compared with 100% mortality in the challenged non-treated fish. Thomas-Jinu & Goodwin (2004) recommended that further testing is needed to determine if a lower concentration of diquat is effective. The current literature lacks information on the *in vitro* antibacterial sensitivity of *F. columnare* isolates to diquat and on the *in vivo* efficacy of diquat against columnaris infection at concentrations below or above 5.4 mg L^{-1}. The lack of this information makes it a challenge to formulate treatment concentrations of diquat effective against columnaris infection. The objectives of this study were to assess the *in vitro* antibacterial activity of diquat against multiple *F. columnare* isolates by conducting minimum inhibitory concentration (MIC) assays and to evaluate the *in vivo* efficacy of single diquat treatments at different concentrations using an acute *F. columnare* infection model in channel catfish with clinical signs of the disease.

Materials and methods

Bacterial isolates

Flavobacterium columnare ATCC23463 and ATCC49512 were obtained from the American Type Culture Collection (ATCC), Manassas, Virginia. Nineteen *F. columnare* isolates were obtained from Auburn University, Alabama (Dr Joseph Newton, College of Veterinary Medicine; Dr John Grizzle, College of Agriculture). Another two isolates were received from the University of Arkansas at Pine Bluff, Arkansas (Dr Andrew Goodwin, College of Agriculture). The *F. columnare* isolates were presumptively and definitively identified by biochemical phenotype (Griffin 1992) and by polymerase chain reaction (PCR) according to Darwish, Ismaiel, Newton & Tang (2004). The *F. columnare* isolates were also previously genotyped according to Darwish & Ismaiel (2005).

In vitro sensitivity of *Flavobacterium columnare*

The MIC of diquat against *F. columnare* was determined in sterile 96 micro-well plates according to a modified method of Darwish, Farmer and Hawke (2008). *Flavobacterium columnare* isolates were cultured on Ordal's medium (Anacker & Ordal 1959) at 28 °C for 24 h. The bacteria were dislodged with a sterile cotton swab and suspended in 3–4 mL sterile saline (0.85% NaCl solution). The absorbance of the bacterial suspension was adjusted to an optical density (OD) of 0.08–0.1 at 625 nm, equivalent to the turbidity of a 0.5 McFarland suspension or 60–70 nephelometer turbidity units (Miller, Walker, Carson, Coles, Coyne, Dalsgaard, Gieseker, Hsu, Mathers, Papapetropoulou, Petty, Teitzel & Reimschuessel 2005). A 100-μL aliquot of the bacterial saline suspension was inoculated into 12 mL of 1/5 (4 g L^{-1}) diluted Muller Hinton broth (DMHB) to achieve an approximate 5.0×10^5 cfu mL^{-1}. The DMHB with the bacteria was pipetted into sterile 96 micro-well plates; all the wells had 100-μL aliquots except for the first wells, which had 196-μL aliquots. A 10 mg mL^{-1} stock solution of diquat was prepared (1 mL of diquat solution at a concentration of 447 mg mL^{-1} in 44.7 mL of water). The stock solution (4 μL) was added to the first wells containing the 196 μL of DMHB with the bacteria to achieve a final diquat concentration of 20 μg mL^{-1}. Aliquots (100 μL) from the first wells were transferred to the other wells to produce double-fold dilutions to 0.156 μg mL^{-1}. For each bacterial isolate, the MIC assay was conduced in triplicate wells and the plates were incubated at 28 °C for 48 h. The MIC was defined as the minimum diquat concentration that prevented visible bacterial growth of the microorganism. Positive control wells had the bacteria suspended with no diquat added. The purity of the positive control wells was confirmed by streaking on Ordal's medium and by Gram staining.

In vivo efficacy studies

Experimental design

Channel catfish fingerlings (14.25 ± 3.3 g; mean ± standard deviation) were provided by the Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas. Using a randomization table generated by MINITAB program version 13 (MINITAB Inc., State College, PA, USA), groups of 10 fish each were randomly allocated to 30 continuously aerated flow-through tanks containing 60 L of well water; two groups per tank, 20 fish per tank. The flow-through tank system provided well water at a flow rate of approximately 1 L min^{-1} and the temperature was maintained at 26.3 ± 0.6 °C. Fish were acclimatized to the experimental conditions for 10 days. The 30 tanks were randomly assigned to one of six treatment groups (5 tanks per treatment): (i) a group of fish challenged by cutaneous scrubbing and waterborne exposure to *F. columnare*, and not treated with diquat (positive control); (ii) four treatment groups of challenged fish treated (at 22-h post-challenge for 6 h) with diquat active ingredient at 2.5, 5.0, 10.0 or 15 mg L^{-1}; (iii) a non-challenged group treated with 15 mg L^{-1} at the same time and duration (negative control). The fish were fed a commercial CCF feed (Arkat Feed Co., Inc.) during the acclimatization period and throughout the course of the experiment, except for the day before the challenge and the day of the challenge.

Dissolved oxygen (> 6 mg L^{-1}) and total ammonia nitrogen (< 0.2 mg L^{-1}) were measured in six tanks daily (HACH DR/2010, HACH Chemical Co.). Six different tanks were sampled on each day so that within 5 days all 30 tanks were tested; this was repeated throughout the experiment.

Infection protocol

Flavobacterium columnare (isolate LV359-01) was stored at −80 °C in Hsu-Shotts broth (with 25% glycerin added) until needed. The bacterium was plated on Ordal's medium (Anacker & Ordal 1959). An isolated colony was used to inoculate a 5-mL start up medium of *F. columnare* growth medium (FCGM) broth (Farmer 2004). The start up medium was incubated for 24 h at 28 °C and used to inoculate 1 L of FCGM broth. The broth was incubated for 24 h at 28 °C with orbital shaking of 200 revolutions per minute (New Brunswick Scientific). The purity of the culture was confirmed by streaking on an Ordal's medium plate. At the end of the acclimatization period and prior to the bacterial challenge, fish of all treatment groups were cutaneously abraded according to Darwish, Mitchell & Straus (in press). The scrubbed area of the skin was the right lateral surface of the fish from the caudal end of the adipose fin to the base of the caudal fin. The bacterial challenge was initiated by placing the 20 cutaneously abraded fish from each tank in a bucket containing 8 L of aerated water and adding 87 mL of the FCGM broth having an OD of 0.85 at 550 nm. After 2 h of the bacterial challenge, the fish were removed from the challenge buckets and returned to their respective tanks. The negative controls were similarly handled, but not exposed to *F. columnare*. The experimental protocol complied with the Animal Care and Use Committee of the Harry K. Dupree-Stuttgart National Aquaculture Research Center.

Necropsies were performed on dead and moribund fish. Bacterial isolations were obtained from skin lesions and trunk kidneys using plates of selective Ordal's medium (Hawke & Thune 1992). The necropsies and bacterial isolations were also performed at the conclusion of the experiment (21 days post-challenge) on four fish sacrificed from each tank, except when fewer than five fish were left in a tank in which case all of the fish were sampled. The identity of isolated bacteria was confirmed by PCR (Darwish *et al.* 2004) and genotyped according to Darwish *et al.* (2005).

Diquat dosing

After 22-h post-challenge water flow was turned off to all tanks. Volumes of diquat stock solution (447.01 g L^{-1}) of 336, 671, 1342 and 2013 μL were added to the 60 L tanks to achieve 2.5, 5.0, 10.0 and 15 mg L^{-1} final diquat concentrations, respectively. The non-challenged group (negative control) was treated with diquat at 15 mg L^{-1} while the challenged group (positive control) was not treated with diquat. Upon dosing the tanks, three of five tanks in each treatment were randomly sampled. Each 500 mL water sample was collected in an amber glass bottle, refrigerated for 2 days and delivered via a cooler with ice packs to Cornerstone Laboratories, LLC for diquat analysis (Hodgeson, Bashe & Eichelberger 1992). At the conclusion of the 6-h treatment with diquat, the water flow was restored in all tanks.

Statistical analysis

At the conclusion of the experiment, the survival percentages within tanks were arcsine transformed and using the MINITAB program version 13 the transformed data were subjected to one-way analysis of variance (Zar 1984; Sokal & Rohlf 1995). The assumptions of ANOVA of the transformed data were tested using MINITAB; the Kolmogorov–Smirnov test confirmed normality, and Bartlett's test confirmed homogeneity of variances. The differences among treatment means were determined by Tukey's procedure (Tukey 1953). Treatment effects were considered significant at $P \leq 0.05$.

The GRAPHPAD PRISM version 4 (GraphPad Software, Inc.) was used to conduct the following survival analysis; (i) Kaplan–Meier method to calculate the probability of survival to each day, and (ii) logrank tests to compare whether the survival curves were significantly different from each other (Motulsky 1995). A Bonferroni correction of P/n, where $P \leq 0.05$ and n = the number of pair-wise comparisons, was employed to account for increasing Type I error with the number of comparisons made (Motulsky 1995).

Results

In vitro sensitivity of *Flavobacterium columnare*

Fifteen of the 23 *F. columnare* isolates had a MIC for diquat of 5.0 mg mL^{-1} while the rest of the isolates had MIC < 5 mg mL^{-1} (Table 1).

Table 1 The minimum inhibitory concentration (μg mL^{-1}) of diquat for *Flavobacterium columnare* isolates tested in 1/5 (4 g L^{-1}) diluted Muller Hinton broth at 28 °C after 44–48 h of incubation

Isolate	Diquat (μg mL^{-1})
Genotype I	
ATCC 23463[a]	5.0
ATCC49512	0.312
L90-268	0.312
Evan 2	1.25
Genotype II	
LV359-01	5.0
L90-639	5.0
L90-629	5.0
L90-640	1.25
LV339-01	5.0
L88-173	5.0
90-503	5.0
Ga-6-93	5.0
064-93	5.0
Genotype III	
AU-98-24	5.0
143-94	5.0
97-01	5.0
90-106	1.25
L90-659	5.0
90-497	1.25
90-498	0.625
ALG 92-509A	5.0
Dickerson I	5.0
L91-20	0.156

[a] *Flavobacterium columnare* type strain.

In vivo efficacy

Diquat reduced the mortality of channel catfish experimentally challenged with *F. columnare*. There was a significant difference between the final mortality of challenged non-treated fish and challenged fish treated with diquat at ≥5 mg L^{-1}. The increase in the diquat dose from 5 to 10 and to 15 mg L^{-1} reduced the final mortality of challenged fish, but the decrease was not statistically significant (Table 2).

According to the logrank comparison the survival curves of the 5, 10 and 15 mg L^{-1} treatments were not significantly different from each other. The survival curve of the 5 mg L^{-1} was not significantly different from the 2.5 mg L^{-1}, but was significantly different from the positive control. The 2.5 mg L^{-1} survival curve was significantly different from the survival curves of the positive control and treatments ≥10 mg L^{-1} (Fig. 1, Table 3).

Severe infection was evident by the 95% mortality in the challenged non-treated fish and 90% bacterial isolation from dead and moribund fish; the infection was systemic with 80% isolation from the kidney or both kidney and cutaneous lesions and was external with only 10% isolation from cutaneous lesions. The identity and genotype of the *F. columnare* isolated was confirmed. No *F. columnare* was isolated from non-challenged fish and from sacrificed fish at the conclusion of the study, 21 days post-challenge.

Clinical signs and gross pathology

The non-challenged negative control treated with diquat appeared normal with no notable clinical signs. In moribund fish, the area of the skin scrubbed prior to the bacterial challenge developed a focal ulcerative necrotizing dermatitis surrounded by cutaneous depigmentation. As the infection progressed, challenged fish had discrete and diffuse, multifocal, skin depigmentation lesions, often

Table 2 Final percent mortality (mean ± standard error) of channel catfish challenged by waterborne exposure to *Flavobacterium columnare* after cutaneous abrasion, and treated once at 22-h post-challenge for 6 h with diquat at 0, 2.5, 5, 10 and 15 mg L^{-1}. Means followed by a different letter were significantly different ($P < 0.05$). Each treatment had five tanks with 20 fish in each tank (100 fish/treatment)

	Diquat dose level (mg L^{-1})					
	0	2.5	5	10	15	Control[1]
Mortality % within tanks	95 ± 3^{c}	74 ± 8^{bc}	68 ± 5^{b}	59 ± 7^{b}	49 ± 14^{b}	0 ± 0^{a}

[1]Negative control, non-challenged fish treated with 15 mg L^{-1} diquat.

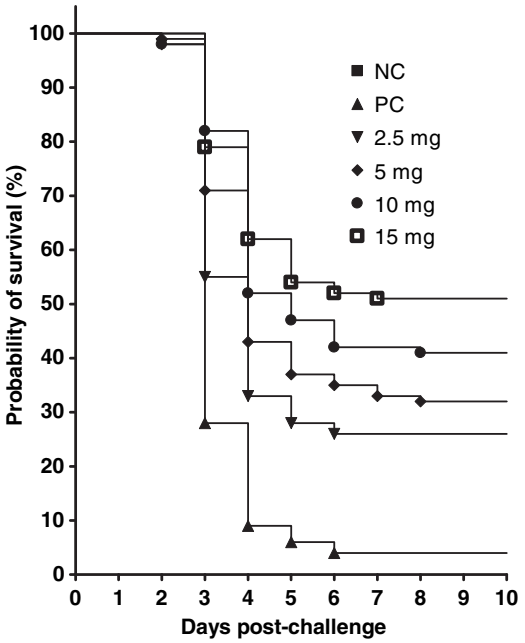

Figure 1 Survival curves showing the daily survival probability of channel catfish challenged by cutaneous abrasion and waterborne exposure to *Flavobacterium columnare* and treated with diquat. The experiment had six treatments; challenged fish not treated with diquat used as a positive control (PC), challenged fish treated at 22-h post-challenge for 6 h with diquat at 2.5, 5.0, 10.0 and 15 mg L^{-1} and a non-challenged group treated at the same time and duration with 15 mg L^{-1} diquat used as negative control (NC). Each treatment had 100 fish equally divided among five tanks.

encompassing most of the body, and the muscles exhibited severe necrotizing myositis. The fins were frayed and necrotic. The buccal mucosa was yellow tinged. The gills had multifocal, necrotizing branchitis with haemorrhages and yellowish mucoid material. Moribund fish were lethargic. No visceral gross pathology was observed.

Diquat dosing

The measured diquat concentrations were 14.8 ± 0.42 mg L^{-1}, 14.3 ± 0.46 mg L^{-1}, 10.6 ± 0.3 mg L^{-1}, $4.6 \pm .27$ mg L^{-1} and 2.3 ± 0.53 mg L^{-1} for the negative control and the 15, 10, 5 and 2.5 mg L^{-1} treatments, respectively.

Discussion

This study examined the *in vitro* sensitivity of *F. columnare* to diquat and the efficacy of a single diquat application at different concentrations against experimental columnaris infection. *In vitro*, the isolates had a MIC value of ≤5 μg L^{-1} and diquat significantly reduced the mortality in treated fish.

Efficacious bath treatments against columnaris will be advantageous compared with feeding medicated feed as infected fish cease to feed at early stages of the infection (Plumb 1999). In the study of Thomas-Jinu & Goodwin (2004), diquat was the most effective bath treatment against columnaris; it produced 100% survival compared with 25% and 31% for chloramine-T and potassium permanganate, respectively.

A comprehensive evaluation of a therapeutant considers several factors including: (i) the concentration; (ii) the frequency and duration of the treatment; (iii) the disease model employed and (iv) the pathogenesis stage at which the treatment is applied. The current study evaluated one treatment at different doses, but did not evaluate repeated treatments. Thomas-Jinu & Goodwin (2004) examined one indefinite treatment at one dose. The disease model in the current study and the one used by Thomas-Jinu & Goodwin (2004) are distinctly different. Fish in the current model were compromised by cutaneous scrubbing and the duration of the bacterial challenge was 2 h compared with the continuous challenge of uncompromised fish in the model of Thomas-Jinu & Goodwin (2004). The disease model in the current study yielded clinical signs very similar to a natural infection and 95% mortalities occurring over

Table 3 Logrank comparison of the survival curves and the hazard ratios of channel catfish challenged by waterborne exposure to *Flavobacterium columnare* after cutaneous abrasion, and treated at 22-h post-challenge for 6 h with diquat at a dose of 0, 2.5, 5, 10 and 15 mg L^{-1}. Challenged fish not treated with diquat (0 mg L^{-1}) were a positive control. The treatments intersect has the *P* value (top)/ hazard ratio (bottom) of the two survival curves compared. The *P* value is the probability that two compared curves are identical in the overall population. The hazard ratio compares the slope of the two survival curves-a hazard ratio of 2 indicates that rate of death in one group is twice the rate in another group

Diquat treatment, mg L^{-1}	Negative control[a]	0 mg L^{-1}	2.5 mg L^{-1}	5 mg L^{-1}	10 mg L^{-1}
0	$P < 0.0001$*				
	NA[3]				
2.5	$P < 0.0001$*	$P < 0.0001$*			
	NA	1.63			
5	$P < 0.0001$*	$P < 0.0001$*	$P = 0.1198$		
	NA	2.07	1.24		
10	$P < 0.0001$*	$P < 0.0001$*	$P = 0.0021$*	$P = 0.1148$	
	NA	2.58	1.56	1.27	
15	$P < 0.0001$*	$P < 0.0001$*	$P < 0.0001$*	$P = 0.0064$	$P = 0.2213$
	NA	2.96	1.87	1.56	1.23

[a]The negative control was non-challenged fish treated with 15 mg L^{-1} diquat and had no mortalities (slope of zero).
*Using the Bonferroni correction the *P* value was significant at $P \leq 0.0033$. The Bonferroni correction of *P*/n, where $P \leq 0.05$ and n = the number of pair-wise comparisons, was employed to account for increasing Type I error with the number of comparisons made.

8 days compared with 100% mortality within 48 h in Thomas-Jinu & Goodwin (2004). The current study used a flow through system except for the duration of the treatment compared with the static system used by Thomas-Jinu & Goodwin (2004). Bacterial numbers would be expected to build up more in a static system than a flow through system. In the static system used by Thomas-Jinu & Goodwin (2004), bacteria would probably be abundant in the water as well as colonized on the fish. Treatments killing bacteria colonized on the fish would be considered therapeutic while those killing bacteria in the water would be considered prophylactic. It is certain that diquat has a prophylactic effect based on the *in vitro* testing performed. The current study's model primarily tested the therapeutic effect of diquat, but it was not possible to distinguish between the therapeutic and prophylactic potential of the diquat treatment in the Thomas-Jinu & Goodwin (2004) model. The different models could account for different efficacies in the two studies. It is likely that the Thomas-Jinu & Goodwin (2004) study would have tested in part the prophylactic effect of diquat.

Based on our *in vitro* study results diquat would be of value as a prophylactic treatment. The uncompromised fish, the indefinite treatment and the prophylactic effect of diquat in Thomas-Jinu & Goodwin (2004) study can partially explain the zero mortality at 5.4 mg L^{-1} compared with the higher mortalities of the current study of 74 ± 8%, 68 ± 5%, 59 ± 7% and 49 ± 14% at diquat concentrations of 2.5, 5.0, 10 and 15 mg L^{-1} for 6 h, respectively. During a natural disease outbreak fish will probably exhibit the infection at different pathogenesis stages and some fish in the population may not be infected; therefore, a treatment encompassing both therapeutic and prophylactic efficacy will be advantageous. Treatment results from the flow through system in the current experiment will have more application to tank cultures while the static indefinite treatment of Thomas-Jinu & Goodwin (2004) will have more application to ponds.

The MIC values of the *F. columnare* isolates tested in the study were ≤5 μg mL^{-1}. The MIC of the *F. columnare* isolate used in the infection was 5 mg L^{-1}, so it is likely that the *in vivo* efficacy in the current experiment will apply to other infections having isolates with MIC of ≤5 μg mL^{-1}. The 5 mg L^{-1} MIC of the isolate used in the infection could partially explain the final lower mortality in the treatments dosed at ≥5 mg L^{-1}, but not at 2.5 mg L^{-1}. It is also likely that infections with more sensitive isolates of *F. columnare* can be effectively treated with diquat concentrations < 5 mg L^{-1}.

The gross pathology of columnaris is often external, involving the gills, skin and underlying muscles with no noticeable visceral lesions (Plumb 1999; Darwish, Mitchell & Hobbs 2008; Darwish, Mitchell & Straus in press), but the infection is

often systemic, as in the current experiment (Hawke & Thune 1992). The efficacy in the current infection was probably caused by the antibacterial effect of diquat on bacteria that have colonized on external surfaces. Diquat has strong cationic properties, which would result in low permeability and absorption in gill epithelia and skin. Channel catfish exposed to 5 and 20 μg mL^{-1} for 5 h had diquat concentrations in the plasma of 0.0056 and 0.0182 μg g^{-1}, respectively, and the highest concentration in any organ was in the trunk kidney at 0.0934 and 0.351 μg g^{-1}, respectively (Schultz, Hayton & Kemmenoe 1995). Schultz *et al.* (1995) also reported that channel fish exposed to diquat for 24 h at 5 μg mL^{-1} had diquat concentrations of 0.0351 and 1.44 μg g^{-1} in the plasma and trunk kidney, respectively. The 6-h diquat treatment in the current study is relatively close to the 5-h treatment of Schultz *et al.* (1995). Considering the difference between the plasma and trunk kidney concentrations reported above, and the 5 μg mL^{-1} MIC of the *F. columnare* isolate used in the challenge, it is highly unlikely that diquat had a significant antibacterial effect on systemic bacteria. For an antibacterial agent to be effective systemically the plasma concentration needs to exceed the MIC of the pathogen by a factor of 2–4 (Blood, Henderson & Radostits 1979).

Disease models are useful research tools that simulate a natural infection, but duplicating a natural infection will always remain a challenge because of the sheer complexity of the factors that produce infections in nature. In natural infections, individual fish in a population are likely to exhibit columnaris at different severity levels and at different pathogenesis stages and some individuals may not be infected (Darwish *et al.* 2008; Darwish, Mitchell & Straus in press). These conditions are difficult to produce experimentally and the efficacy of diquat in a natural infection may differ from that found in the current experiment. In experimental infections, challenge conditions are usually kept consistent to produce a fish population at a similar pathogenesis stage and infection severity. This distinct difference between natural and experimental infections must be considered carefully in assessing experimental efficacy and highlights the importance of field efficacy studies.

Diquat is an Environmental Protection Agency-approved herbicide for use in food fish cultures (Plumb 1999) and has the potential to be legally and practically used against acute columnaris. The application of diquat in tank culture rather than ponds would be more advantageous because of: (i) the prohibitive economic cost in ponds (Thomas-Jinu & Goodwin 2004) and (ii) the probable reduction of efficacy because of diquat binding to organic matter in ponds (Schultz *et al.* 1995). Our study results in tanks with limited organic matter might be an underestimate of the diquat rate needed in ponds for effective treatment. Also, the current study tested the efficacy of diquat against columnaris using one application, but it is unclear if repeated applications can increase columnaris survival. Further research will be warranted to test diquat efficacy in ponds and to optimize dose concentration, application frequency and duration.

Acknowledgements

The authors would like to acknowledge Bradley Farmer for his superior and dedicated technical assistance throughout the course of the study. The authors are also grateful for the excellent reviews provided by Dr Kevin Schrader, Dr Michael Mauel, Dr Jerry Ludwig and Dr Julie Bebak. Mention of trade names of commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Received: 4 September 2008
Revision received: 18 November 2008
Accepted: 25 November 2008